

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

B-1

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶: A01N 37/02, 37/06, 37/10, A23K 3/03, A23L 3/3517	A1	(11) International Publication Number: WO 96/24248 (43) International Publication Date: 15 August 1996 (15.08.96)
(21) International Application Number: PCT/FI96/00066 (22) International Filing Date: 5 February 1996 (05.02.96) (30) Priority Data: 950512 6 February 1995 (06.02.95) FI (71) Applicant (for all designated States except US): KEMIRA CHEMICALS OY [FI/FI]; Porkkalankatu 3, FIN-00180 Helsinki (FI). (72) Inventors; and (75) Inventors/Applicants (for US only): AHLNÄS, Thomas [FI/FI]; Mäntytie 7 A 9, FIN-00270 Helsinki (FI). MÜLLER, Arthur [SE/SE]; Bärnstenagatan 19 c, S-253 61 Helsingborg (SE). MALMQVIST, Sven-Olof [SE/SE]; Tranekärr 8586, S-263 92 Jonstorp (SE). LÖFGREN, Timo [FI/FI]; Hanikka 32 A, FIN-02360 Espoo (FI). OLLIKAINEN, Terttu, Inkeri [FI/FI]; Kivenhakkajantie 9 D, FIN-04200 Kerava (FI). (74) Agent: BERGGREN OY AB; P.O. Box 16, FIN-00101 Helsinki (FI).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>In English translation (filed in Finnish).</i>
(54) Title: ANTIMICROBIC COMPOSITION AND ITS USE (57) Abstract <p>The invention is related to an antimicrobial composition whose antimicrobial component contains: a) about 50-99.8 weight % of unsubstituted or substituted C₁-C₄ monocarboxylic acid, and b) about 0.2-30 weight % of the ester or unsubstituted or substituted benzoic acid. The composite of the carboxylic acid and the ester of benzoic acid yields better results than when using, e.g., benzoic acid. The composition according to the invention can also contain a carrier in particle form in which the said antimicrobial component has been absorbed.</p>		

BEST AVAILABLE COPY

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

ANTIMICROBIC COMPOSITION AND ITS USE

The invention is related to an antimicrobial composition and its use as a substance
5 inhibiting the growth of detrimental microbes.

One significant quality-improving factor in food stuffs and in beef cattle and poultry
forage is that they are free from *Salmonella*, *Listeria*, *E. coli* and other bacteria
which cause food poisoning. Abundant use of antibiotics in controlling bacteria is
10 not recommended because it results in the growth of resistant bacterial populations.
Therefore, it is desired to discover improved preservatives.

According to the publication *Poultry International*, July 1994, pp. 40-42, mixtures
of organic acids, particularly propionic acid and formic acid, or mixtures of organic
15 acids and their ammonium salts, or salts of organic acids are used to prevent
Salmonella in poultry forage. The products are either solutions or solids.

The publication *Applied and Environmental Microbiology*, Aug. 1980, 352-357
discloses that benzoates and their derivatives have been widely used as
20 antimicrobials. Their effect increases with increased acidity, implying that the free
acid works as an active ingredient.

The effect of propionic acid, acetic acid, sorbic acid, and benzoic acid and mixtures
thereof are known from mould control of moist feed grain (*Poultry Science* 60
25 (1981), 2182-2188).

The publication *Food Microbiology*, 5 (1988) : 3, pp. 135-139 explains that a
composition containing 1 % acetic acid, 1 % lactic acid, maleic acid, tartaric acid,
or citric acid, 0.05 % sorbic acid, 0.05 % benzoic acid, and 3.5 % salt, acts, in a
30 salad whose pH is buffered to 4.8, as a bactericide with respect to *Salmonella*
blockley, *Escherichia coli*, and *Staphylococcus aureus*, and is bacteriostatic with
respect to *Streptococcus Faecalis*.

The publication WO 92/21239 discloses an antimicrobial composition against, e.g.,
35 *Salmonella*, containing a polyunsaturated C₅-C₁₄ monocarboxylic acid, such as
sorbic acid or its derivative, together with a substituted or an unsubstituted aromatic
carboxylic acid, such as benzoic acid or its salt or ester. The compositions may also

contain organic or inorganic acids. Formic acid is not presented among the numerous acids listed in the publication.

The publication International Journal of Food Microbiology 22 (1994) : 2-3, pp. 127-140, explains that the composition of potassium sorbate and sodium benzoate slows down or inhibits the growth of *Salmonella* in milk or cheese when its pH is regulated with propionic acid.

Esters of parahydroxylbenzoic acid, such as propylparaben, possibly together with butylated hydroxyanisole, are known to have an inhibiting effect on the growth of *Salmonella* (Proc. Int. Symp. Food Microbiol., 11th Meeting 1980, 377-84 (1981); J. Food Prot. (1980), 43(3), 191-4).

These prior art antimicrobial compositions have the disadvantage that when benzoic acid is used, its migration to microbe cells is unsatisfactory. On the other hand, when an ester of benzoic acid is used, its migration to microbe cells is satisfactory but it is not as effective as benzoic acid as an antimicrobial.

The problem of the publications described above has now been solved with the aid of a new antimicrobial composition which is mainly characterised in comprising an antimicrobial component that contains:

- a) about 50-99.8 weight-% of unsubstituted or substituted C₁-C₄ monocarboxylic acid, and
- b) about 0.2-30 weight-% of an ester of an unsubstituted or a substituted benzoic acid.

By using organic acid and an ester of benzoic acid together, a mixture is provided which penetrates microbe cells and forms an effective antimicrobial benzoic acid in the microbe cells. The present invention is related to compositions according to this principle in particular.

Thus it has been discovered that compositions containing C₁-C₄ monocarboxylic acid and esters of unsubstituted or substituted benzoic acid are effective in preventing *Salmonella* and other detrimental microbes and inhibiting the growth thereof.

The antimicrobial composition according to the invention comprises a first antimicrobial component consisting of a first subcomponent a) which is an

- unsubstituted or substituted C₁-C₄ monocarboxylic acid. The preferred amount is about 60-98 weight-% of the weight of the antimicrobial component. According to one embodiment, the unsubstituted or substituted C₁-C₄ monocarboxylic acid is an acid selected from: formic acid, acetic acid, propionic acid, n-butyric acid, isobutyric acid, glycolic acid, lactic acid, hydroxybutyric acid, pyruvic acid, acetacetic acid, and a mixture thereof, preferably formic acid, acetic acid, propionic acid, lactic acid or a mixture thereof, most preferably formic acid or a mixture of formic acid and propionic acid.
- 10 The antimicrobial composition according to the invention comprises an antimicrobial component consisting of a second component which is an ester of an unsubstituted or substituted benzoic acid. The antimicrobial component preferably contains about 1-20 weight-%, and preferably about 2-10 weight-% of an ester of unsubstituted or substituted benzoic acid. According to one preferred embodiment, the ester of the
- 15 unsubstituted or the substituted benzoic acid is selected from esters of an unsubstituted benzoic acid and from those of a benzoic acid substituted with one or more alkyls, halogens, nitros, hydroxies, and/or aminos. Especially preferred esters comprise esters of benzoic acid or those of p-hydroxybenzoic acid.
- 20 According to one embodiment, the ester of the unsubstituted or the substituted benzoic acid is the ester of the unsubstituted or substituted benzoic acid and an alcohol which is selected from: benzoic alcohol, methanol, ethanol, n-propanol, isopropanol, n-butyl alcohol, and isobutyl alcohol. Especially preferred ester-forming alcohols include methanol, ethanol, n-propanol, and isopropanol.
- 25 Alcohols containing several hydroxyl groups that are viable for the ester of benzoic acid of the invention include, e.g., glycols, such as 1,2-propanediol, and triols, such as glycerol.
- 30 Particularly preferred esters include methyl benzoate, ethyl benzoate, n-propyl benzoate, isopropyl benzoate, methyl-p-hydroxylic benzoate, ethyl-p-hydroxylic benzoate, n-propyl-p-hydroxylic benzoate, isopropyl-p-hydroxylic benzoate, and butyl-p-hydroxylic benzoate.
- 35 The results of a laboratory test carried out indicate that the effect of the mixtures of carboxylic acid/benzoic acid esters according to the invention is at its best if the stability of the esters against hydrolysis has been suitably lowered. It has been discovered in particular that the restarting of the growth of *Salmonella* after the

treatment has been decreased in this case. Migration in forage material and through the cell walls of bacteria is probably strongest when the ester form is prevailing but the correctly-timed decomposition of esters and the resulting benzoic acid generated *in situ* in the object of use might be significant with respect to the effect of the
5 bacterium.

It is important that, if in addition to said C_1 - C_4 monocarboxylic acid component, another substance that accelerates the hydrolysis of the ester of the unsubstituted or the substituted benzoic acid is used. The preferred content of this substance is 0.2-
10 20 weight-% of the weight of the antimicrobial component. A preferable content is 3-15 weight-%.

The advantageous embodiments of the invention thus contain activators that accelerate the decomposition of the ester. Tests have also indicated that
15 compositions that catalyze the decomposition of esters, can also decompose carboxylic acid, formic acid in particular. Typical activators include mineral acids, such as sulphuric acid, hydrochloric acid, nitric acid, and phosphoric acid or a mixture thereof. Regarding mineral acids, hydrochloric acid is a stronger catalyser than nitric acid.

20 According to one embodiment, the antimicrobial component of the composition according to the invention also comprises about 0.02-20 weight-%, preferably about 0.5-10 weight-% of the ester of C_1 - C_4 monocarboxylic acid that acts synergistically with the ester of the unsubstituted or the substituted benzoic acid, with C_1 - C_4
25 alcohol. Typical such esters include methylester, ethylester or propylester of formic acid, acetic acid or propionic acid. These substances are preferably obtained when C_1 - C_4 alcohol, preferably propanol, is added to the composition *in situ*.

In addition to the above-mentioned ingredients, the antimicrobial component may
30 contain other compositions that improve the preservation power, such as C_4 - C_{14} carboxylic acids, such as free benzoic acid, isobutyric acid or octanoic acid, or corresponding alcohols; monoglycerides of C_8 - C_{16} carboxylic acids or other bactericidal or bacteriostatic surface-active compositions, dicarboxylic acids, such as oxalic acid, malonic acid, succinic acid, adipic acid, pimelic acid, suberic acid,
35 fumaric acid, or maleic acid; polykarboxylic acids, such as polyacrylic acid, or copolymeric acids formed between unsaturated compounds that are polymerised with acrylic acid and polyacrylic acid; hydroxycarboxylic acids, dicarboxylic acids or polycarboxylic acids, such as sorbic acid, ascorbic acid, cinnamic acid, salicylic

acid, tartaric acid or maleic acid; complexing agents, such as EDTA, DTPA; or derivatives, such as salts or esters, of all the above-mentioned acids. The mixture may, depending on the application of use, further contain salts affecting as preservatives, including salts of magnesium, zinc and copper, compositions that
5 adjust animal nutrients or the salt content of the mixture or that buffer its pH, including ammonium phosphate or potassium sulphate; imidiazolindylurea, phenolic compositions and sulphonic acids, such as lignosulphonates, or aldehydes, such as formadehyde. Furthermore, components acting as antioxidants, such as butylated hydroxyanisole or hydroxytoluene, or esters of gallic acid can be added to
10 the mixture.

In addition to C_1 - C_4 monocarboxylic acids, the compositions according to the invention can also contain other compounds that react with water in an acidly manner, such as organic or inorganic acids or polyacids, or compounds that form
15 acids in a solution. The acids may also be in a partly neutralised form, such as ammonium salts, for instance. The compositions that form acids include inorganic or organic salts or acid anhydrides that react in an acidly manner.

Since even relatively small amounts of water decrease and, correspondingly,
20 activators increase the decomposition of both esters and formic acid, the properties of the mixture can be modified so that a suitable level of activity and a storage stability sufficient for each application are achieved. The freezing point of the antimicrobial liquid component of the composition according to the invention is a few degrees above freezing point if its C_1 - C_4 acid content is high and the water
25 content very low at the same time. By increasing the water content, the melting point can be lowered considerably when desired. A preferred water content is about 1-30 weight-% and preferably about 5-20 weight-%.

In conventional temperatures, the preservatives according to the invention are either
30 in the form of a solution or in a solid form. The advantages of the solid state include better security during transport, a slight smell, and an easy dispensing. According to a preferred embodiment of the invention, the composition comprises, in addition to the antimicrobial component, also a solid corpuscular carrier. In this case, it is advantageous if the weight ratio between the antimicrobial component and the
35 corpuscular carrier is in the range of 1:10 - 10:1, preferably in the range of 3:7 - 7:3. Because the issue often is about absorbing the antimicrobial liquid component on the corpuscular carrier, the specific surface of the corpuscular carrier has to be large enough, preferably at least about 10, and more preferably at least about 100 m²/g.

The approximate particle size of the corpuscular carrier is preferably 0.01-20 mm, most preferably about 0.1-2 mm.

The corpuscular substance mentioned above is preferably selected from the following: inorganic corpuscular substances based on silicium dioxide and/or alumina, such as siliceous earth, moler earth, silicic acid, silica, alumina, bentonite, montmorillonite, perlite, steatite, chlorite, expanded vermiculite, calcium silicate, sodium aluminium silicate, and zeolites, inorganic salts that dissolve sparingly in water and organic acids, and organic substances such as synthetic polymers, cellulose, cellulose derivatives, lignin and lignin derivatives.

The solid composition according to the invention ranges from fairly dry to fairly moist. The former is easy to dispense also in lower temperatures, the latter may cake together in temperatures lower than the melting point of the liquid phase but when the temperature is increased, it is again easy to process. The processibility can be even improved by powdering with a fine solidifying agent or other powder.

As already mentioned before, the composition according to the invention comprises an antimicrobial substance that is preferably absorbed in the corpuscular substance. Particularly good preservative fluids include those that are easy to handle by dispensing devices, such as granular mineral absorbents, including moler earth, and lumpy celluloses.

Solic compositions are thus prepared by absorbing a composition in liquid form as such in a large-area inorganic or organic absorbent, such as silica, clay mineral, or cellulose or mixtures thereof. Solid compositions may also be prepared so that its liquid ingredients or liquid compositions of the ingredients are separately absorbed in similar or different absorbents, which are finally mixed together. At the same time, other possible solid ingredients may be added. The composition according to a particularly preferred embodiment of the invention is in a solid particle-like form so that the mineral acids and other possible compositions that unstabilize formic acid or the ester of the unsubstituted or substituted benzoic acid are situated in different particles than the formic acid and the said benzoic acid esters.

Thus the antimicrobial substance is absorbed in the first part of the corpuscular substance, and the substance that accelerates the hydrolysis of the ester of the unsubstituted or the substituted benzoic acid is absorbed in the second part of the corpuscular substance.

A particularly preferred embodiment of the antimicrobial composition according to the invention is obtained with a composition comprising:

- 1) an antimicrobial component comprising:
 - 5 a) about 50-99.8 weight-% of unsubstituted or substituted C₁-C₄ monocarboxylic acid,
 - b) about 0.2-30 weight-% of the unsubstituted or the substituted ester of benzoic acid,
 - c) about 0-20 weight-% of a substance that causes a hydrolysis of the ester of the
10 unsubstituted or the substituted benzoic acid,
 - d) about 0-20 weight-% of the ester of C₁-C₄ monocarboxylic acid with alcohol that acts synergistically with the ester of the unsubstituted or the substituted benzoic acid,
 - e) about 0-30 weight-% of water, and
- 15 2) a solid corpuscular carrier, whereby the weight ratio between the antimicrobial component and the solid corpuscular substance is in the range of 1:10 - 10:0.

This preferred embodiment also comprises the advantageous components and parametric relations which are presented above separately.

20 Lastly, the invention is related to the use of any of the compositions described above as a substance inhibiting the growth of hazardous microbes, such as *Salmonella*, in forage material and forage products and the like. In this case, it is possible to add the composition to the forage material or animal forage in the form
25 of a processing material or an additive. The composition may also be used to preserve green forage or grain, to improve the hygiene of cattle sheds and the like, or to disinfect devices, equipment, and packages.

30 Some implementive and reference examples are described in the following, the sole purpose thereof being to illustrate the invention.

Example 1 FORAGE TEST

An inoculate of *Salmonella infantis*, 1500-3000 bacteria/g of forage, was evenly mixed with a commercial forage product for feeding chickens, including about 2/3
35 of grain, the rest being mainly fish meal and soya. The studied preservative was spread in the infected forage as evenly as possible in an amount corresponding to a dosage of 8 kg/t. Peptone water was measured into a control bottle. The cultures were kept in 1 litre Erlenmayer bottles sealed with aluminium foil in room

temperature. 2 parallel samples of 10 g each were taken of the cultures each sampling day. Sets of dilution were made of the samples after an Ultra-Turrax mixing. 50 µl of the samples were spread from the dilution tubes on agar sheets on Petri dishes. The bacterial colonies formed after an incubation of 24 h/37° C were counted.

Example	Preservative	pH	The time for the Salmonella to disappear (2 parallel determinations)
10		0d/42d	
Comparison	Peptone water	6.8/6.5	42 d, 42 d
Comparison	Solution 1 *)	6.2/5.8	7 d, 7 d
Comparison	95% solution 1	6.0/5.8	5 d, 42 d
15	5% H ₂ SO ₄		
Comparison	90% solution 1	5.9/5.8	7 d, 7 d
	10% H ₂ SO ₄		
Invention	85% solution 1	6.0/6.1	1 d, 2 d
20	10% H ₂ SO ₄		
	5% ethyl benzoate		

*) 78 % formic acid, 2 % phosphoric acid, and 20 % water

Example 2 A test was conducted as in the previous example but using another forage sample. The following results were obtained:

Example	Preservative	pH	The time for the Salmonella to disappear (2 parallel determinations)
30			
Comparison	Peptone water	6.5	19 d, 30 d
Comparison	Solution 1 *)	5.9	19 d, 19 d
Invention	85% solution 1	6.1	5 d, 5 d
35	10% HCl		
	5% ethyl benzoate		

*) 78% formic acid, 2 % phosphoric acid, and 20% water

The examples show that the forage buffers the test solution so that no result is achieved in the preservative through decrease in pH.

Example 3 SALMONELLA

- 5 The *Salmonella infantis* used in the test had originally been isolated from a chicken and cultivated in buffered peptone water. For the test, sets of dilution were made in peptone water by using different amounts of preservatives. The growth of bacteria was observed by a Bioscreen device (Labsystems, Finland). The device automatically measures, from the set of samples, the cloudiness that depicts
10 bacterial growth as a function of time. The results are presented in Figs. 1 (acetic acid) and 2 (acetic acid with 2.5 weight-% of EB and 2.5 weight-% of propyl formate). It is discernible that the growth of Salmonella is inhibited when the acetic acid content is 0.5 weight-%, but when 5 weight-% of the 1:1 ester mixture of ethyl benzoate + propyl formate according to the invention was mixed with the acetic
15 acid, the growth could be inhibited even with 0.3 weight-% of preservative.

Example 4A THE EFFECT OF MINERAL ACID ON STABILITY

- The effect of sulphuric acid and hydrochloric acid on the stability of liquid compositions was studied. About 10 g of each composition was weighed into 100
20 ml bottles which were tightly sealed and kept in an increased temperature of 45° C for 10 days to accelerate the reaction. Then the amount of carbon monoxide gas generated in the gas phase of the bottles during the decomposition of the formic acid and the amount of the/ ethyl benzoate left in the solution and that of the benzoic acid generated as its breakdown product were analyzed. The results are shown
25 below:

Composition (weight fractions)		H ₂ SO ₄	HCl
5	Formic acid (99 %)	72.6	72.6
	Sulphuric acid (37 %)	23.1	-
	Hydrochloric acid (37 %)	-	23.1
	Ethyl benzoate	4.3	4.3
10	After one week:		
	Appearance	a clear solution	crystalline
	CO, ml/bottle *)		
	a)	0.91	25.8
15	b)	0.91	26.0
	Ethyl benzoate, %		
	a)	0.90	0.08
	b)	0.90	0.07
	Benzoic acid, %		
20	a)	2.73	3.51
	b)	2.71	3.38

*) a) and b) are parallel samples

- 25 It is discernable that both acids activate the decomposition of both the ester and the formic acid. When hydrochloric acid was used, a considerable amount of carbon monoxide gas had formed already during one week in an incubator, and the amount of benzoic acid was so large that some of it had crystallized. This composition that is rich in hydrochloric acid is thus suitable to be used only when no preservation
- 30 time is required of the product.

Example 4B

- The test of Example 4A was repeated so that propyl benzoate was used instead of ethyl benzoate. The amount of propyl benzoate in the sample containing sulphuric
- 35 acid was 1.68 weight fractions, and 0.14 weight fractions in the sample containing hydrochloric acid. Both solutions remained clear.

Example 5 STABILITY TEST ON A SOLID FORMULA: STRONG FORMIC ACID AND AN 85 % FORMIC ACID

- 40 Liquid mixtures were absorbed in a solid absorbent according to the next table. The obtained products were packed in impermeable bottles which were kept in increased

temperatures. The amount of carbon monoxide generated as a breakdown product of the formic acid was analyzed from the gas phase of the bottles by using gas chromatograph. The following results were obtained:

5	Composition, weight fractions	A	B	C	D	E	F
	Solid absorbent	60	60	60	60	60	60
	Formic acid (99 %)	40	-	34	-	38	
10	Formic acid (85 %)	-	40	-	34	-	38
	H ₂ SO ₄ , strong	-	-	4	4	-	2
	Ethyl benzoate	-	-	2	2	2	2
.....							
	The amount of the formed CO, ml/100 g						
15	15 h in 60° C	0.2	0.1	3.8	0.3	-	-
	36 h in 45° C	-	-	-	-	0.1	0.1

20 The test indicates that with sulphuric acid present, the 99 % formic acid starts to dehydrate but aqueous formic acid is distinctly more stabile.

Example 6 PREPARATION OF SOLID ABSORBENT

25 59 weight fractions of granular absorbent called Damolin (Dansk Moler Industri A/S) with a particle size of 0.2-0.6 mm was dispensed to the forward end of a screw conveyor during a certain period of time by using a feeding device. During the same period of time, 41 weight fractions of an acid mixture was fed on top of the absorbent on the screw conveyor by using a pump. The acid mixture contained:

	Formic acid	68.0 weight-%
30	Water	15.3 weight-%
	Sulphuric acid	10.0 weight-%
	Ethyl benzoate	5.0 weight-%
	Phosphoric acid	1.7 weight-%

35 A fixed product was obtained which was easy to dispense.

Example 7 PREPARATION OF SOLID ABSORBENTS

The following fixed mixtures were made:

		7/1	7/2	7/3	7/4
5	FA:EB 95:5	40	48	70	70
	Damolin	60	48	30	-
	Zeothix 265	-	4	-	-
	Cellulose	-	-	-	30

- 10 FA = Formic acid
EB = Ethyl benzoate

Damolin was the same as the one used in the previous example. Zeothix 265 is a precipitated silicic acid (Huber Corporation). The cellulose was commercial sheet
15 cellulose which was used to make pieces of about 1mm x 1mm by shredding it first with a shredder to form strips, which were then fed in transversally and, finally, oversized pieces were screened out.

Mixture 7/1 was granular and easy to process. Mixture 7/2 felt dry but did not flow
20 as easily as the former mixture. Mixture 7/3 was fine and dry, but vault-forming, and thus more difficult to dispense. Mixture 7/4 maintained its block-like form and remained relatively easy to process, even though it felt damp. The hardness of its pieces could be further improved by powdering the mixture with small amounts of a salt-like substance which form a slightly soluble formate such as calcium or
25 magnesium formiates. Such substances include, e.g., finely powdered calcium chloride, calcium carbonate, dolomite, and calcium sulphate.

Example 8 MINERAL ACID IN THE SAME/A DIFFERENT GRANULE

60 parts by weight of a granular absorbent was mixed with 40 parts by weight of a
30 mixture of strong formic acid, sulphuric acid, and ethyl benzoate in a ratio of 85:10:5. Another mixture was made with a similar approximate composition by mixing together a) 90 parts by weight of a mixture containing 60 weight-% of the absorbent and 40 weight-% of a mixture of formic acid and ethyl benzoate in a ratio of 94.4:5.6, and b) 10 parts by weight of a mixture containing 40 weight-% of
35 sulphuric acid and 60 weight-% of the absorbent. About 10 g samples of the mixtures were weighed into 100 ml bottles which were closed tightly and placed into an incubator at a temperature of 45° C for one week. The amount of carbon monoxide gas generated in the gas phase of the bottles in the breakdown reaction of

formic acid was then analyzed. The results indicate that the stability of the product can be improved considerably by placing the mineral acid in a different granule:

5	Sample	CO, ml/sample
	Mineral acid in the same granule with formic acid and a benzoic acid ester	31.1
10	Mineral acid in a different granule	1.34

Example 9 PREPARATION OF A SYNERGISTIC ESTER MIXTURE

Compositions according to the following table were prepared:

15

	Example	9/A	9/B	9/C	9/D
	FA (EB)	95	85	85	75
20	H ₂ SO ₄	-	-	10	10
	Water	-	10	-	10
	Propanol	5	5	5	5

25 FA(EB) : a mixture with 93.9 parts by weight of formic acid and 4.1 parts by weight of ethyl benzoate, and 0.03 parts by weight of benzoic acid.

When the samples were analyzed, it could be perceived that none of the mixtures no longer contained propanol. Instead, propyl formiate had been formed:

30

		9/A	9/B	9/C	9/D
	Formic acid	87.5	75.4	76.5	66.7
35	Ethyl benzoate	4.1	3.6	3.0	2.4
	Benzoic acid	0.03	0.03	0.5	0.6
	Propanol	0.0	0.0	0.0	0.0
	Propyl formiate	7.3	7.0	6.8	6.7

40

In a corresponding manner, it was possible to create propyl acetate in preservatives with acetic acid, and propyl propionate in solutions with propionic acid.

CLAIMS

1. An antimicrobial composition, characterised in comprising an antimicrobial component containing
 - 5 a) about 50-99.8 weight-% of an unsubstituted or a substituted C_1 - C_4 monocarboxylic acid, and
 - b) about 0.2-30 weight-% of the ester of an unsubstituted or a substituted benzoic acid.
- 10 2. A composition according to Claim 1, characterised in that the antimicrobial component comprises about 60-98 weight-% of an unsubstituted or a substituted C_1 - C_4 monocarboxylic acid.
- 15 3. A composition according to Claim 1 or 2, characterised in that the unsubstituted or the substituted C_1 - C_4 monocarboxylic acid is an acid selected from the following: formic acid, acetic acid, propionic acid, n-butyric acid, isobutyric acid, glycolic acid, lactic acid, hydroxybutyric acid, pyruvic acid, acetacetic acid, and a mixture thereof, preferably formic acid, acetic acid, propionic acid, lactic acid, or a mixture thereof, preferably formic acid or a mixture of formic acid and propionic acid.
- 20 4. A composition according to Claim 1, 2 or 3, characterised in that the antimicrobial component comprises about 1-20 weight-%, preferably about 2-10 weight-% of the ester of an unsubstituted or a substituted benzoic acid.
- 25 5. A composition according to any of the preceding Claims, characterised in that the ester of the unsubstituted or the substituted benzoic acid is selected from the esters of a benzoic acid that is unsubstituted and a benzoic acid that is substituted by one or more alkyls, halogens, nitros, hydroxies, and/or aminos.
- 30 6. A composition according to Claim 5, characterised in that the the ester of the unsubstituted or the substituted benzoic acid is selected from the esters of benzoic acid or p-hydroxybenzoic acid.
- 35 7. A composition according to any of the preceding Claims, characterised in that the ester of the unsubstituted or the substituted benzoic acid is the ester of the unsubstituted or the substituted benzoic acid and an alcohol that is selected from the following: benzoic alcohol, methanol, ethanol, n-propanol, isopropyl alcohol, n-butyl alcohol, isobutyl alcohol.

8. A composition according to any of the preceding Claims, characterised in that the ester of the unsubstituted or the substituted benzoic acid is selected from the following: methyl benzoate, ethyl benzoate, n-propyl benzoate, iso propyl benzoate, methyl-p-hydroxybenzoate, ethyl-p-hydroxybenzoate, n-propyl-p-hydroxybenzoate, isopropyl-p-hydroxybenzoate, and butyl-p-hydroxybenzoate.
9. A composition according to any of the preceding Claims, characterised in that the antimicrobial component comprises
- c) about 0.2-20, preferably 3-15 weight-% of a substance that accelerates the hydrolysis of the ester of the unsubstituted or the substituted benzoic acid.
10. A composition according to Claim 9, characterised in that the substance that accelerates the hydrolysis of the ester of the benzoic acid is a mineral acid or a mineral acid mixture.
11. A composition according to any of the preceding Claims, characterised in that the antimicrobial component comprises
- d) about 0.02-20 weight-%, preferably about 0.5-10 weight-% of the ester of C₁-C₄ monocarboxylic acid with C₁-C₄ alcohol, affecting synergistically with the ester of the unsubstituted or the substituted benzoic acid.
12. A composition according to Claim 11, characterised in that the said ester of C₁-C₄ monocarboxylic acid with C₁-C₄ alcohol is the methyl ester, the ethyl ester or the propyl ester of formic acid, acetic acid or propionic acid.
13. A method for preparing the ester composition according to Claim 11 or 12, characterised in that the component d) is allowed to be formed in the composition by adding C₁-C₄ alcohol, preferably propanol, to it.
14. A composition according to any of the preceding Claims, characterised in that its antimicrobial component contains about 1-30 weight-%, preferably about 5-20 weight-% of water.
15. A composition according to any of the preceding Claims, characterised in comprising a fixed carrier in particle form.

16. A composition according to Claim 15, characterised in that the weight ratio between the antimicrobial component and the carrier in particle form is in the range of 1:10 - 10:1, preferably in the range of 3:7 - 7:3.
- 5 17. A composition according to Claim 15 or 16, characterised in that the specific surface of the carrier in particle form is at least about 10, preferably at least 100 m²/g.
- 10 18. A composition according to Claim 15, 16 or 17, characterised in that the approximate particle size of the carrier in particle form is 0.01-20, preferably 0.1-2 mm.
- 15 19. A composition according to any of Claims 15-18, characterised in that the corpuscular substance is selected from the following: inorganic corpuscular substances based on silicium dioxide and/or aluminas, including siliceous earth, moler earth, silicic acid, silica, alumina, bentonite, montmorillonite, perlite, steatite, chlorite, expanded vermiculite, calcium silicate, and zeolites, inorganic salts slightly soluble in water and organic acids, and organic substances such as synthetic polymers, cellulose, cellulose derivatives, lignin, and ligneous derivatives.
- 20 20. A composition according to any of Claims 15-19, characterised in that the antimicrobial substance is absorbed in the corpuscular substance.
- 25 21. A composition according to any of Claims 15-20, characterised in that the antimicrobial substance is absorbed in the first part of the corpuscular substance and the substance that generates the hydrolysis of the ester of the unsubstituted or the substituted benzoic acid is absorbed in the second part of the corpuscular substance.
- 30 22. A composition according to any of the preceding Claims, characterised in comprising:
1) an antimicrobial component containing:
a) about 50-99.8 weight-% of unsubstituted or substituted C₁-C₄ monocarboxylic acid,
b) about 0.2-30 weight-% of unsubstituted or substituted ester of benzoic acid,
35 c) about 0-20 weight-% of a substance generating the hydrolysis of the ester of the unsubstituted or the substituted benzoic acid,

- d) about 0-20 weight-% of the ester of C₁-C₄ monocarboxylic acid with alcohol, affecting synergistically with the ester of the unsubstituted or the substituted benzoic acid,
- e) about 0-30 weight-% of water, and
- 5 2) a fixed carrier in particle form so that the weight ratio between the antimicrobial component and the solid carrier in particle form substance is in the range of 1:10 - 10:0.
- 10 23. The use of the composition according to any of the preceding Claims as a substance that inhibits the growth of hazardous microbes, such as *Salmonella*, in forage materials and preparations, and similar materials.
- 15 24. The use according to Claim 23 as a processing matter or an additive of forage material or animal forage.
25. The use according to Claim 23 for preserving green forage or grain, for improving the hygiene of cattle sheds and similar premises, or for disinfecting devices, equipment, and packages.

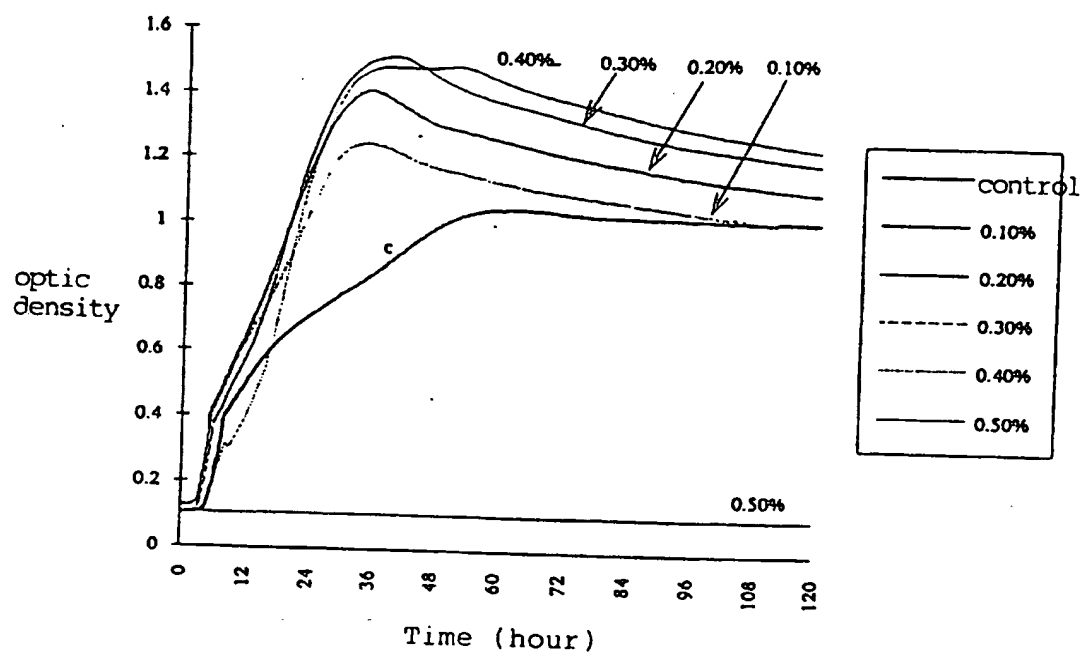
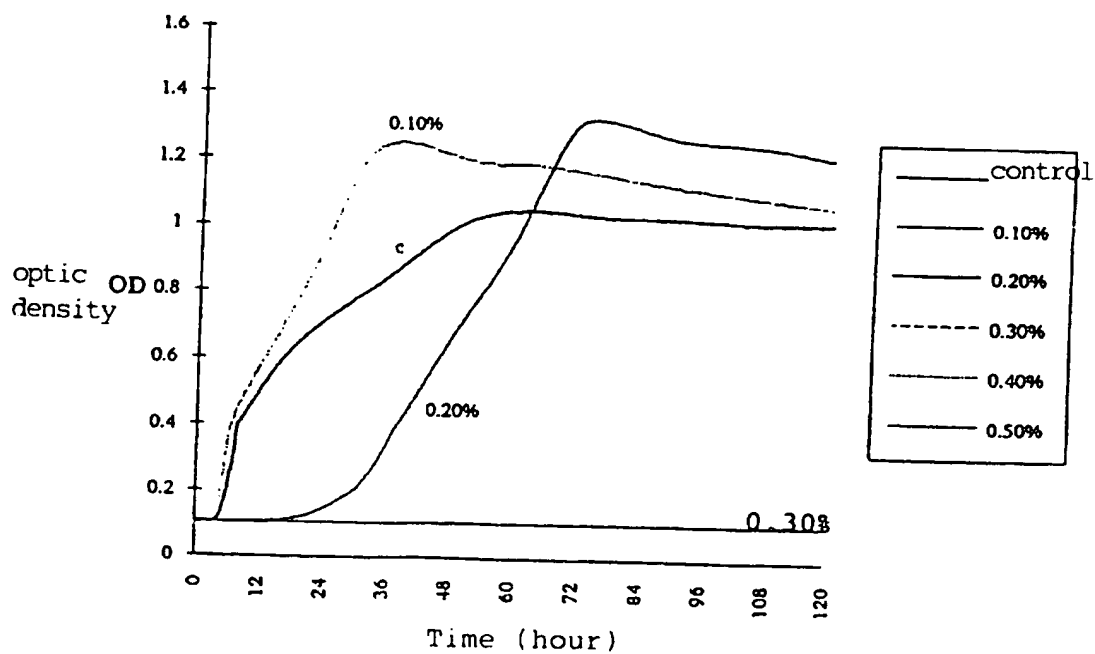
1/1
AS 5

FIG. 1 Acetic acid

AS 11

FIG. 2 Acetic acid + 2,5 % EB + 2,5 % PF
(EB = ethyl benzoate, PF = propyl formiate)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00066

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A01N 37/02, A01N 37/06, A01N 37/10, A23K 3/03, A23L 3/3517

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A01N, A23K, A23L, A23B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9316611 A1 (KEMIRA OY), 2 Sept 1993 (02.09.93) --	1-25
X	WO 9221239 A1 (ECOLAB INC.), 10 December 1992 (10.12.92) --	1-25
X	WO 9316603 A1 (KEMIRA OY), 2 Sept 1993 (02.09.93) --	1-25
X	GB 2095534 A (MARTTI EMIL LAMPILA), 6 October 1982 (06.10.82) --	1-25

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 May 1996

Date of mailing of the international search report

07 -05- 1996

Name and mailing address of the ISA/
 Swedish Patent Office
 Box 5055, S-102 42 STOCKHOLM
 Facsimile No. +46 8 666 02 86

Authorized officer

Gerd Strandell

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/FI 96/00066

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DE 2507067 A (PLATE KOFASIL GMBH), 2 Sept 1976 (02.09.76) -----	1-25

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/FI 96/00066

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 9316611	02/09/93	AU-A- 3502193	13/09/93
		EP-A- 0626812	07/12/94
		FI-B, C- 95195	29/09/95
		LT-A- 706	31/01/95
		LT-B- 3474	25/10/95
		LV-B- 10563	20/02/96
		NO-A, D- 943168	26/08/94
WO-A1- 9221239	10/12/92	AU-B- 665548	11/01/96
		AU-A- 2001192	08/01/93
		EP-A- 0588912	30/03/94
		JP-T- 6508612	29/09/94
		NZ-A- 240355	27/09/94
		US-A- 5336500	09/08/94
		US-A- 5419908	30/05/95
WO-A1- 9316603	02/09/93	AU-A- 3502293	13/09/93
		LT-A- 362	15/07/94
GB-A- 2095534	06/10/82	DE-A, C, C 3208134	18/11/82
		FR-A, B, B 2502468	01/10/82
		NL-A- 8200629	18/10/82
		SE-B, C- 454477	09/05/88
		SE-A- 8200625	01/10/82
DE-A- 2507067	02/09/76	AT-B- 344496	25/07/78
		CH-A- 621466	13/02/81
		FR-A, B- 2301181	17/09/76
		GB-A- 1535233	13/12/78
		JP-C- 1104505	16/07/82
		JP-A- 51114270	07/10/76
		JP-B- 56050543	30/11/81
		US-A- 4073889	14/02/78

This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☐ ~~FADED~~ TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☐ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.